

§Appl. No. 10/009,500
Amdt. dated August 23, 2004
Reply to Office Action of, April 21, 2004

In the Specification:

Please amend the specification as follows:

On page 25, the second full paragraph has been amended as follows:

Example 16: Preparation of Immunoglobin from Immune Sera of Rabbits
(anti-ConA, anti-hemoglobin and anti-peptide rabbit antibodies)

The rabbit sera were raised with the use of the following immunogens: concanavalin A lectin, mixture of hemoglobins and peptide-KLH conjugates. The peptide sequence was identical with that of the 14 amino acid N-terminal part of manillase (KEIAVTIDDKNVIA).

The sera were purified on the Protein A Sepharose (Pharmacia, 17-0780-01) column according to the standard Pharmacia instruction. The purity of the IgG samples were checked with the aid of SDS-PAGE and ELISA test.

Example 16: - Preparation of Immunoglobin from Immune Sera of Rabbits
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On page 28, the first full paragraph has been amended as follows:

Example 24: Construction of the *E. coli* Expression Vector (Fig. 11)

For expression in *E. coli* we used a modified version of the plasmid pASK75, which carries the tet promoter region. (Skerra, Gene 151, (1994), pp 131-135). The modification we made by cloning a new linker between the XbaI an Hind III sites. The new linker contains the ompA leader sequence, another multiple cloning site and a 6xHis tag instead of the strep tag. Linkersequence which was cloned in pASK75.

XbaI
119 CTAGATAACG AGGGCAAAAA ATGAAAAAAGA CAGCTATCGC GATTGCAGTG GCACCTGGCTG
TATTGC TCCCGTTTT TACTTTTCT GTCGATAGCG CTAACGTCAC CGTGACCGAC
1► Met Lys Lys T hrAla IleAl aIleAl aVal Al aLeuAl aG
Clal EcoRI SstI KpnI SmaI BamHI
179 GTTTCGCTAC CGTAGCCGAG GC AT CGA TGA ATT CGA GCT CGG TAC CCG GGG
CAAAGCCATG CCATGCCGTG CG TA GCT ACT TAA GCT CGA GCC ATG GGC CCC
14► IyPheAlaThr rValAlaGln Al a
Xhol SalI PstI Eco47III
230 ATC CCT CGA GGT CGA CCT GCA GGC AGC GCT ATGAGAGGATCGCATCACCATCACCA
TAG GGA GCT CCA GCT GGA CGT CCG TCG CGATACTCTCTAGCGTAGTGGTAGTGGT
Hind III 1► Al aMet Ar g Gl y Ser Hi s Hi s Hi s Hi
286 TCACTAATAGA
AGTGATTATCTTCGA
10► sHi s

Example 24: - Construction of the *E. coli* Expression Vector (Fig. 11)

For expression in *E. coli* we used a modified version of the plasmid pASK75, which carries the tet promoter region. (Skerra, Gene 151, (1994), pp 131-135). The modification we made by cloning a new linker between the XbaI an Hind III sites. The new linker contains the ompA leader sequence, another multiple cloning site and a 6xHis-tag (SEQ ID NO: 17) instead of the strep-tag.

Linkersequence which was cloned in pASK75. (SEQ ID NOS 18-20)

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XbaI
119 CTAGATAACG AGGGCAAAAA ATGAAAAAAGA CAGCTATCGC GATTGCAGTG GCACTGGCTG
TATTGC TCCCGTTTT TACTTTTCT GTCGATAGCG CTAACGTAC CGTGACCGAC
1► Met Lys Lys T hrAla IleAl aIleAl aVal Al aLeuAl aG
Clal EcoRI SalI KpnI SmaI BamHI
179 GTTTCGGTAC CGTAGCCGAG GC AT CGA TGA ATT CGA GCT CCG TAC CCG GGG
CAAAGCGATG GCATCGCGTC CG TA GCT ACT TAA GCT CGA GCC ATG GGC CCC
14► IyPheAlaTh rValAlaGln Al a
Xhol SalI PstI Eco47III
230 ATC CCT CGA GGT CGA CCT CGA GGC AGC GCTATGAGAGGATCGCATCACCATCACCA
TAG GGA GCT CCA GCT GGA CGT CCG TCG CGATACCTCCCTAGCGTAGTGGTAGTGGT
Hind III 1► Al aMet Ar g Gl y Ser Hi s Hi s Hi s Hi
286 TCACTAATAGA
AGT GATTATCTTCGA
10► sHi s.....

On page 28, the second full paragraph has been amended as follows:

~~To construct the expression vector for manillase it was necessary to introduce 5' Cla I and 3'~~
~~Eco47III restriction sites by PCR method. Therefore the two primers 5' ATC GAT AAA GAG~~
~~ATT GCC GTG AC and~~
~~3' GTT GTT TCC GAT GCT AAA GCG CT~~
~~were used. The PCR product first was cloned into the PCR II vector system~~
~~(Invitrogen) and sequenced.~~

To construct the expression vector for manillase it was necessary to introduce 5' Cla I and 3'
Eco47III restriction sites by PCR method. Therefore the two primers 5' ATC GAT AAA GAG
ATT GCC GTG AC (SEQ ID NO: 8)and
3' GTT GTT TCC GAT GCT AAA GCG CT (SEQ ID NO: 9)
were used. The PCR product first was cloned into the PCR II vector system (Invitrogen) and
sequenced.

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On page 28, the fourth full paragraph has been amended as follows:

~~After expressing and proving the activity of this recombinant manillase in a second PCR reaction the His-tag was removed and the start codon of the manillase gene was directly fused to the omp A leader sequence. The primers for this PCR reaction were:~~

~~5' ACC GTA GCG CAG GCC AAA GAG ATT GCC GTG and
3' CAC GGC AAT CTC TTT GGC CTG CGC TAC GGT.~~

After expressing and proving the activity of this recombinant manillase in a second PCR reaction the His-tag was removed and the start codon of the manillase gene was directly fused to the omp A leader sequence. The primers for this PCR reaction were:

5' ACC GTA GCG CAG GCC AAA GAG ATT GCC GTG (SEQ ID NO: 10) and
3' CAC GGC AAT CTC TTT GGC CTG CGC TAC GGT (SEQ ID NO: 11).

The last paragraph bridging pages 28 and 29 has been amended as follows:

Example 25: Construction of the Baculo Donor Plasmid (Fig. 12)
~~For expression of manillase in the Baculo virus expression system the Bac-To-BacTM Baculovirus Expression System from Gibco Life Technologies was used. To get a section system the Honeybee melitin leader sequence was fused to the manillase gene and to introduce the restriction sites 5' BamHI and 3' KpnI one single PCR reaction was carried out.~~

5' Primer:

~~CGG ATC CAT GAA ATT CTT AGT CAA CGT TGC CCT TGT TTT TAT GGT CGT ATA
CAT TTC TTA CAT CTA TGC GAA AGA GAT TGC CGT GAC~~

3' Primer:

~~AAT GTT GAA GCA TAA GGT ACC~~

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Example 25: - Construction of the Baculo Donor Plasmid (Fig. 12)

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Baculovirus Expression System from Gibco Life Technologies was used. To get a section system the Honeybee melitin leader sequence was fused to the manillase gene and to introduce the restriction sites 5' BamHI and 3' KpnI one single PCR reaction was carried out.

5' Primer:

CGG ATC CAT GAA ATT CTT AGT CAA CGT TGC CCT TGT TTT TAT GGT CGT ATA
CAT TTC TTA CAT CTA TGC GAA AGA GAT TGC CGT GAC (SEQ ID NO: 12)

3' Primer:

AAT GTT GAA GCA TAA GGT ACC (SEQ ID NO: 13)

On page 29, the second full paragraph has been amended as follows:

Example 26: - Construction of the Yeast Expression Vector (Fig. 13)

For expression in yeast we used the pichia multi copy expression system (Invitrogen). To construct the expression vector for manillase we used the PCR amplification method of the manillase gene in such a way that compatible restriction ends (5' EcoR I, 3' Not I) are generated for ligation into the appropriate vector (pPIC9K). Therefore the following primers were used:

5' GTA GAA TTC AAA GAG ATT GCC GTG ACA

3' GAT GCT AAT GTT GAA GCA TAA TGA GCG GCC GC

Before transforming the Pichia Speroplasts the expression vector has to be linearized with Sal I.

Example 26: - Construction of the Yeast Expression Vector (Fig. 13)

For expression in yeast we used the pichia multi copy expression system (Invitrogen). To construct the expression vector for manillase we used the PCR amplification method of the

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manillase gene in such a way that compatible restriction ends (5' EcoR I, 3' Not I) are generated for ligation into the appropriate vector (pPIC9K). Therefore the following primers were used:
5' GTA GAA TTC AAA GAG ATT GCC GTG ACA (SEQ ID NO: 14)
3' GAT GCT AAT GTT GAA GCA TAA TGA GCG GCC GC (SEQ ID NO: 15)
Before transforming the Pichia Speroplasts the expression vector has to be linearized with Sal I.